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OBSERVATIONS ON BACILLUS MESENTERICUS AND ALLIED ORGANISMS.*

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THE MAIN TYPES OF THE SUBTILIS GROUP.

The frequency of finding distinct variations in the morphology and cultural characters of the organisms commonly classed as *B. subtilis* led to the study of as many of these forms as could be obtained, with the object of determining if there were any particular relations between them, or any constancy in their characters.

The number of spore-bearing organisms which possess feeble or no pathogenic properties is considerable. In the literature there occur at least 43 distinct species. Of these, however, a considerable proportion must be regarded as mere variants of one or other well-marked types, even though they were originally found in habitats quite disconnected with a bacteriological laboratory.

It is convenient to divide the organisms of this class into four or perhaps five standard types. These types are respectively the bacillus subtilis, the bacillus megatherium (De Bary), the bacillus vulgatus (Flügge), the bacillus mesentericus (Flügge), and possibly the bacillus ellenbachensis (Stutzer).

Morphology of these types.—*B. subtilis*, as is well known, varies considerably in its appearance, though the typical forms are square-ended rods. It is usually smaller than *B. anthracis*, but otherwise very similar. The spores, however, though central, are either of the same diameter or a little larger than the bacillus. Other forms occur in which the bacillus is shorter and thicker, and either square-ended or rounded at the ends. In such cases the spore occupies nearly two-thirds of the length of the organism, and is conspicuously broader. Other forms again occur, apparently identical in cultural reactions, which are still shorter, and usually show a very

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characteristic form owing to the large size of the spore, which then appears to have a cap upon each pole corresponding to the remains of the original bacillus.

B. megatherium differs from the preceding in its slender form, invariably with square ends, and in frequently having a narrow elongated spore toward one pole. The spore is not central, but its center is at some point between the center of the bacillus and its pole. In a few instances the spore is quite terminal, and when its diameter is larger than that of the bacillus, the whole structure acquires a form not unlike that of the bacillus of tetanus, and very like that of the bacillus of malignant edema. As a rule the spore is of exactly the same diameter as the bacillus which bears it. This form may show a granular structure—a false beading—when stained by Gram's method.

B. vulgatus parades under several other names. *B. mesentericus vulgatus* is agreed to be the same organism, and it has also been called the potato bacillus. This organism is long and slender, and either square-ended or rounded at the ends. Except that long thrixlike forms may occur, and that it is more slender than the preceding, the characters of the two are practically identical.

B. mesentericus presents variations of morphology which on the one hand may be subtiliform, and on the other, megatheriform. As a rule it occurs as a short bacillus with rounded ends and a central spore which is broader than the organism and oval in shape. A form is quite common in which the spore is so large that the bacillus itself is reduced to a cap at each end.

The Ellenbach bacillus goes by a number of other names, having been described variously by different authors (Burchard, Frankland, Flügge, Kern, Russell). The ends are rounded, and there are numerous clear spots in the length of the bacillus. It is as large as or larger than the anthrax bacillus, and the oval spores are larger compared with it. It may be arbitrary to describe it thus as a separate main type, but the beaded appearance is so characteristic a feature that it may be emphasized in this way. In the course of our work we have come across gram-positive organisms which were regarded by others as diphtheroid, until we were able to demonstrate the spore formation.

Attached to each of these types there are numerous species described in the literature. They may be arranged as follows:

Under *B. subtilis*: *B. bernesis*, *B. carotarum*, *B. simplex*, *B. sessilis*, *B. leptosporus*, *B. peptonificans*, *B. tenuis*, *B. alerrimus*, *B. implexus*, *B. loxosporus*, *B. natans*, *B. vacuolus*.

Under *B. ellenbachensis*: *B. petroselini*, *B. cereus*, *B. limosus*, *B. lutulentus*, *B. cursor*, *B. loxosus*, *B. turgescens*, *B. stoloniferus*, (*B. robur*).

Under *B. megatherium*: *B. petasites*, *B. silvaticus*, *B. hessii*, *B. quercifolius*, *B. lactis*, *B. pumilus*, *B. leplodermis*.

Under *B. vulgaris*: *B. graveolens*, *B. butyricus*, *B. tumescens*, *B. gummosus*, *B. levaniformis*, *B. gelatinosus betae*, *B. spongiosus*, *B. geniculatus*, *B. granulosus*, *B. lactis*, *B. lacticola*, *B. goniosporus*, *B. lacteus*, *B. aureus*, *B. cylindrosporus*, *B. amarificans*, *B. agglomeratus*, *B. lutulentus*, *B. sphericus*, *B. gracilis*, *B. pseudotetanicus*, *B. albuminis*.

Under *B. mesentericus*: *B. mesentericus ruber*, *B. teres*, *B. filiformis*, *B. liodermos*, *B. pumilus*, *B. ruminatus*, *B. albolactis*, *B. tomentosum*, *B. pansini*, *B. mucosus* (Zimmermann).

THE VARIOUS FORMS OF GROWTH UPON AGAR AND POTATO.

For the purpose of introducing clearness into a difficult group we may arrange the above organisms according as they fall into the types named, but in doing so it must be conceded that the morphology becomes the sole guide, and that this, along with the cultural characters, may be rendered unreliable owing to the variability which is so frequent in individual species. Different critics could easily arrange the various species differently. It is more simple to divide the organisms into *three* groups according to the mode of action upon *sugar media* (see below), but in view of the characteristic appearances presented by the slope cultures (potato or agar) a critical study of such points may be dealt with first, considering that Lehmann and Neumann regarded it best to classify these *subtilis* forms according to the growth on potato.

The *growths upon agar*, as studied in this laboratory, may be divided into these well-marked forms:

a) A white growth which spreads more or less rapidly over the surface of the agar, so that within 48 hours the whole is covered. In this form the growing margin is leaflike and acanthiform. The surface is dry, and frequently has a powdery appearance, as if dusted over with flour. The growth is opaque. In one or two

instances the growth was scanty, dead chalk-white in color, and slow of development (cretaceous form).

Examples—Powdery growth: *B. subtilis*, *B. megatherium*, *B. butyricus*, *B. oxalaticus*, *B. parvus*.

b) A moist creamlike growth which is rapid and very abundant. This form tends to undergo pigmentation, developing a creamy-brown color in some instances, a fawn color in others, and a brilliant ocher-yellow color in others.

c) A gummy transparent growth, which is very easily identified. This form has exactly the appearance of a thin film of ice lying over an opaque milky deposit. This icelike or glassy surface is striking.

Examples of b and c—Smooth growth: *B. mesentericus*, *B. bernensis*, *B. tumescens*, *B. granulatus*, *B. petasites*, *B. asterosporus*, *B. pumilus*, *B. simplex*.

d) A fawn-colored, opaque, dry, rather scanty growth. This form may show fine polygonal depressions or "reticulations" of its surface, the general growth being flat. The older portions of this growth are powdery in appearance.

Examples—Reticulate growth: *B. mesentericus ruber*, some forms of *B. mesentericus*, some forms of *B. megatherium*.

e) A wrinkled growth. These wrinkles occur in various forms, passing in different examples from a fine polygonal, fawn-colored, slightly raised deposit (see *d*), through a more abundant and more raised fawn-colored growth (see *b*), which covers a large portion of the culture tube (though most marked at the lower end), to an excessively abundant wrinkling, such as occurs in some smegma bacillus cultures, and occasional tubercle bacillus cultures. Strikingly picturesque growths may be produced in such cases. The wrinkling, however, may not be dry, but it may possess a luscious moist appearance, especially when arising in the moist forms of creamy or milky growth. Here the wrinkles are usually much larger, and more widely separated.

Examples—Wrinkled growth: *B. vulgatus*, *B. graveolens*, *B. fusiformis*, *B. teres*, *B. liodermos*, *B. mesentericus*, *B. tenuis*, *B. ellenbachensis*, *B. ruminatus*.

These different variations of aspect are obviously not hard and fast, but there are gradual transitions met with between one and

another. Not only are such transitions found in studying series of specimens from different sources, but the same organism may be seen to undergo changes in the courses of successive planting upon the same medium. Thus, in a form which at the outset possessed a growth like the fourth variant, we were able to demonstrate a change through the second to the third by planting at monthly, or two-monthly, intervals—the organisms having been kept in the incubator for three or four weeks at a time. In four months the organism had developed the third form. In another case, the central parts became like type *b*, while the periphery was still like type *c*. Such changes were noticed in several cases, and in numerous examples we noticed that the particular culture would alter in aspect as the medium became more and more dry, though in such examples the change was evidently due to the increased spore production, which invariably predisposes to a dry, powdery, or dusty appearance.

These remarks will indicate what we wish to emphasize, namely, that cultural characters of the “subtilis group” are not fixed in any of its members, so that it is not necessarily incorrect to call a *B. mesentericus*, *B. subtilis*, or vice versa. There is little real criterion to go upon. This statement, however, requires qualifying by indicating that *sugar reactions* remain fairly constant.

The *growths upon potato* may be classified thus:

a) Pigmented growth. These growths are usually moist and vary from a fawn or yellowish brown through ocher to rose or pink. Such growths are elevated and have a granular surface. Sometimes they are wrinkled. The pigment may require several days for its development.

1. Fawn color: *B. pisiformis* (coliform), *B. asterosporus* (no folds), *B. teres* (dry surface).

2. Pale yellow: *B. luteus*, *B. petasites*.

3. Ocher yellow: *B. ochraceus* (proposed name).

4. Yellowish brown: *B. parvus*.

5. Reddish brown: *B. silvaticus*.

6. Pink or rose: *B. tenuis* (no wrinkling), *B. mesentericus ruber* (wrinkles late).

b) Non-pigmented dry growth. The surface in such cases is

dry and dusty (*B. subtilis*, *B. tenuis*). Other varieties have been found which we propose to name according to their main character: *B. iridescens*, *B. cretaceus communior* (abundant growth), *B. cretaceus infrequens* (scanty growth).

c) Non-pigmented moist growth. The surface may present a gummy appearance, with folds arising (*B. simplex*, *B. ruminatus*) or without folds (*B. liodermos*). The surface may be creamy; in such cases the folds may be much raised (*B. vulgatus*, *B. graveolens*, *B. aterrimus*); in other cases the growth has a reticulated surface (*B. mesentericus*); in others the creamy deposit is quite smooth (*B. pumilus*). The surface may be coliform (*B. oxalaticus*, *B. tumescens*, *B. megatherium*, *B. butyricus*) or dull gray (*B. ellenbachensis*, *B. subtilis*, *B. mesentericus*, *B. ruminatus*).

This method of grouping affords a convenient guide to the identification of some of these organisms, but, as already indicated, is not rigidly adhered to by the respective species.

SUBDIVISION OF THE MEMBERS OF THIS GROUP ACCORDING TO MORPHOLOGY.

The variants in cultural forms would naturally be assumed to go hand in hand with morphological variations, but this is not strictly speaking true. The powdery forms are usually long slender or long thick bacilli, while the gummy forms are usually shorter and rounded. In other words, the former are subtiliform, and the latter mesentericiform. But many of the gummy or glassy forms do show delicate slender rods with terminal or nearly terminal spores, such as occur in megatherium and vulgatus.

The moist creamy forms (*b*) are usually long rods of variable thickness and conform to the megatherium type, though here again we meet with dry wrinkled forms of type *e* conforming to the same morphological type.

Among the chalky forms we have met with two examples which were extremely striking. In one the growth on the agar plate was very beautiful, having a mathematically regular feathery star form, the limbs of the star being gently curved. In another form the growth was very scanty, and possessed an extremely white, opaque,

powdery aspect. Both forms were isolated from the intestinal contents of caterpillars.

Classifying the brown species according to the differences in morphology, we have:

Rods, decidedly longer than broad—square ends: *B. robur* (Meyer and Neide), *B. megatherium*, *B. vulgatus*, *B. carotarum* (Koch), *B. mycoides*, *B. mesentericus ruber*, *B. sphericus*, *B. silvaticus*, *B. parvus*—in order of size.

Rods, decidedly longer than broad—rounded ends: *B. subtilis*, *B. ellenbachensis*, *B. simplex*, *B. petasites* (Meyer and Gottheil), *B. pumilus* (Meyer and Gottheil), *B. bolea* (Schiff-Georgini), *B. ruminatus* (Meyer and Gottheil), *B. teres* (Meyer and Neide), *B. graveolens*, *B. mesentericus*, *B. asterosporus*—in order of size.

Rods, very little longer than broad: cocci-bacillary forms; some *B. teres*, and *B. fusiformis*.

Motility. Gram-staining.—All the forms are gram positive and sluggishly motile by the aid of peritrichous flagella. There are a few exceptions to the latter rule, in that *B. sphericus*, *B. fusiformis*, and *B. asterosporus* are very actively motile, while young cultures of *B. subtilis*, *B. parvus*, *B. silvaticus* are fairly motile, and *B. ellenbachensis*, *B. mesentericus ruber*, *B. tenuis*, *B. implexus*, *B. oxalaticus*, *B. simplex*, *B. carotarum* and *B. ruminatus* are non-motile.

ACTION ON GELATIN AND MILK.

Action on gelatin.—The group reaction is liquefaction after varying periods of time. Some specimens liquefy in three or four days. In our series we have found that some forms do not liquefy within three weeks.

Action on milk.—This is quite typical. It consists in the conversion, within a few hours, of the opaque fluid into a watery translucent or even transparent liquid, with a flocculent yellowish or pale brown precipitate. The fluid turns an ocher or orange or lemon yellow, or, occasionally, a violet, or even blue-black color, which is very striking. Some forms produce acid (*B. gastrophilus*) and some produce a preliminary coagulation—*B. anthracis*, *B. vulgatus* (occasionally), *B. ellenbachensis*, *B. ruminatus*, *B. burnensis*,

B. butyricus, *B. asteroides*, *B. oleae*. In the case of *B. pumilus* and *B. petasites*, coagulation occurs quite late.

VARIATIONS OF ACTIONS ON SUGARS.

Action on sugar media.—Most of the published observations are very incomplete. Dextrose may be fermented with acid production in some instances (*B. tenuis*, *B. bernensis*, *B. teres*, and *B. fusiformis* have no action on dextrose) while lactose is rarely attacked (only by *B. butyricus*). The other sugars have been systematically tested in our series, which includes examples obtained from the intestinal contents of both vertebrates and invertebrates—the snail, various caterpillars, frogs, snakes, perch, bass, rats, and some poultry. In addition, many forms were found in the material spoken of at the time as “Chinese eggs,” besides examples from various samples of well-water and various forms found in the hospital patients.

DEXTROSE: This sugar was invariably fermented, though to varying degrees. Using the Andrade indicator and using an arbitrary color scale in which 100 represents the maximum coloration produced by any organism, the acid formation was represented by figures varying from one to 40. In seven cases an appreciable amount of gas was formed (Nos. 4, 17, 23, 25, 27, 33, 35).

Time relations: As a rule the acidity did not alter after the second day, but in some instances there was a marked increase of acidity on the fourth and fifth days of incubation to be followed by a fall (*B. petasites*, *B. megatherium*, *B. tumescens* [No. 11]). In one case the maximum acidity was on the third day (*B. tumescens* [No. 11]).

LACTOSE: This was invariably unaffected in the organisms classed into groups 1 and 2, but in one or two forms corresponding to the *B. megatherium* type, both acid and gas were produced to a moderate extent, but only after six or seven days' incubation (*B. teres*).

SACCHAROSE: This sugar was unaffected by organisms of the first group. They may be termed *non-saccharose fermenters*. The second group comprises those which invariably showed a fermentation with acid production alone, a change presumably due to inversion

of the saccharose. Gas was not formed. Some of the specimens gave a reaction within a day or two, while others failed to give a reaction within two, or sometimes three, weeks. In the case of a third group the saccharose was fermented with both acid and gas production, though in varying and small amounts.

TABLE 1.
NON-SACCHAROSE FERMENTERS.

Consec. No.	Name of Organism	Source	Dextrose	Lactose	Saccharose	Maltose	Mannite	Levulose	Dextrin
			A G				A G		A G
1	<i>B. subtilis</i>	Canned eggs	3-0	0 0	0 0	0 0	0 0	0 0	—
2	<i>B. subtilis</i>	Human jejunum	3-bubble	0 0	0 0	0 0	0 0	0 0	—
3	<i>B. mesentericus ruber</i>	Caterpillar	25-0	0 0	0 0	0 0	0 0	0 0	5-1
4	<i>B. ochraceus</i>	"	40-25	0 0	0 0	0 0	10-0	0 0	5-15
5	<i>B. megatherium</i>	Bass	5-0	0 0	0 0	0 0	0 0	0 0	—
6	<i>B. gummosus</i>	Snail	35-0	0 0	0 0	0 0	0 0	0 0	—
7	<i>B. gummosus</i>	"	15-0	0 0	0 0	0 0	0 0	Trace-0	—
8	<i>B. ruminatus</i>	Caterpillar	30-0	0 0	0 0	0 0	0 0	0 0	2-0
9	<i>B. ruminatus</i>	"	30-0	0 0	0 0	0 0	0 0	10-15	—
10	<i>B. petasites</i>	Canned eggs	10-0	0 0	0 0	0 0	0 0	0 0	—
11	<i>B. tumescens</i>	Snail	25-0	0 0	0 0	0 0	0 0	0 0	—
12	<i>B. liodermos</i>	Canned eggs	20-0	0 0	0 0	0 0	0 0	0 0	—
13	<i>B. gastrophilus</i>	Hen's egg	10-5	0 0	0 0	0 0	0 0	—	0-1

TABLE 2.
SACCHAROSE FERMENTERS.

Consec. No.	Name of Organism	Source	Dextrose	Lactose	Saccharose	Maltose	Mannite	Levulose	Dulcite	Dextrin
			A G	A G	A G		A G	A G		A G
14	<i>B. mesentericus</i>	Bass intestine	15-0	0 0	5-0	0 0	0 0	—	0 0	—
15	"	Canned egg	15-0	0 0	10-0	0 0	5-0	0 0	0 0	—
16	"	"	5-0	0 0	5-0	0 0	5-0	0 0	0 0	—
17	"	Perch intestine	10-0	0 0	30-15	0 0	20-0	20-10	0 0	—
18	<i>B. cretaceus</i>	Raw sugar	3-0	0 0	5-0	0 0	Trace-0	—	0 0	—
19	<i>B. megatherium</i>	Rat intestine	15-0	0 0	15-0	0 0	0 0	—	0 0	—
20	"	Bass intestine	5-0	0 0	5-0	0 0	0 0	—	0 0	—
21	<i>B. gummosus</i>	Snail intestine	35-0	0 0	35-0	0 0	0 0	—	0 0	—
22	<i>B. teres</i>	Perch intestine	10-10	0 0	Late	0 0	0 0	—	0 0	—
					10-0					
23	<i>B. petasites</i>	Caterpillar	45-25	3-0	3-0	0 0	10-0	—	—	5-15

Time relations: Acid production became marked on the fourth day, and reached its maximum during the fifth and sixth days.

(Examples: *B. megatherium*, *B. mesentericus*, *B. teres*, *B. cretaceus*.)

MALTOSE: This was rarely attacked, but a few variants were found (Nos. 24-31, 33-35) in which a slight degree of acid formation

was noticed. These forms occurred indiscriminately in the three groups referred to. Some megatherium forms produced gas as well as acid.

Time relations: Gas was produced by some in considerable quantity, the maximum formation occurring on the fourth day, though acid was formed in greater and greater quantities from this date onward till the fourteenth day, when it diminished again (*B. megatherium*). As a rule the reactions remained constant from the time at which they first appeared (first day).

MANNITE: The mannite fermenters were very few. Some produced only acid, and some both acid and gas.

Time relations: Acid formation steadily progressed till the 12th day, after which diminution occurred (*B. megatherium* No. 32). As a rule the reactions remained the same throughout as they were on the first day.

Summarizing these sugar reactions for diagnostic purposes, we notice that the first broad subdivision of the members of the group is into (a) fermenters of dextrose; (b) non-fermenters of dextrose.

The second group is the less numerous, including *B. subtilis*, *B. tenuis*, *B. fusiformis*, *B. atterimus*; *B. gummosus*, *B. gastrophilus* may also reasonably fall into this group. It is important to note that *B. subtilis* is much more frequently a non-fermenter than a fermenter and that its action is always minimal, even if present.

The first group may be divided up into single-sugar fermenters or multiple-sugar fermenters, and the latter into saccharose fermenters, mannite fermenters, maltose fermenters, and levulose fermenters.

Multiple fermenters:

a) Saccharose fermenters: *B. megatherium*, *B. mesentericus*, *B. gummosus* (marked), *B. teres* (late), *B. cretaceus* (variable).

b) Mannite fermenters: *B. mesentericus* (some forms), *B. petasites*, *B. ochraceus*.

c) Maltose fermenters: *B. megatherium*, *B. pumilus*, some forms of *B. mesentericus*, *B. gastrophilus*.

d) Levulose fermenters: *B. ruminatus*, some variants of *B. mesentericus*.

The other organisms are all "single-sugar fermenters," and only produce a minimal reaction. *B. mesentericus ruber*, however, acts vigorously.

TABLE 3.
MULTIPLE FERMENTERS.

Consec. No.	Name of Organism	Source	Dextrose		Lactose		Saccharose		Maltose		Mannite		Levulose		Dextrin		Dulcite
			A	G	A	G	A	G	A	G	A	G	A	G	A	G	
24.....	<i>B. megatherium</i>	Bass intestine	5-	0		0	10-15		25-10		0		—		0		0
25.....	"	"	15-15		5-	0	20-10		25-0		0		—		—		0
26.....	"	Snail	20-0		0		0		70-0		0		15-0		—		0
27.....	"	"	10-10		10-10		5-0		15-15		0		—		—		0
28.....	"	Child, feces	5-0		0		15-0		15-0		0		—		—		0
29.....	"	"	10-2		15-0		5-6		2-2		0		—		—		0
30.....	"	Caterpillar	25-3		0		0		2-0		0		—		10-0		0
31.....	"	Bass intestine	3-0		0		0		5-0		0		—		—		0
32.....	"	Snail	10-0		0		0		0		20-0		15-0		—		0
33.....	<i>B. mesentericus</i>	Bass intestine	0		0		0		10-10		5-10		—		—		0
34.....	<i>B. gastrophilus</i>	Canned egg	5-0		10-0		0		5-0		5-0		—		—		0
35.....	<i>B. pumilus</i>	"	0		0		0		75-10		5-0		—		5-0		0

TABLE 4.
NON-FERMENTERS OF DEXTROSE.

- 36.....*B. subtilis*, in six examples
 37.....*B. tenuis*, in three examples
 38.....*B. cretaceus*
 39.....*B. gummosus*
 40.....*B. fusiformis*
 41.....*B. gastrophilus*
 42.....*B. aterrimus*
 43.....*B. bernensis*
 44.....*B. teres*

The occurrence of motile and non-motile forms, the occurrence of milk-coagulators and milk non-coagulators, as well as the presence of morphological variations, prevent the use of the above classification as the main measure of identification of these organisms. It is for this reason that a diagnostic table which combines all such considerations has been devised (see p. 223).

LEVULOSE: Very strong action was noticed with levulose in a number of instances. The medium became strongly acid, and in a few instances gas was also produced. Examples: Nos. 7, 9, 17, 26, 32.

Time relations: This was often late, the change commencing on the fourth day and reaching a maximum on the seventh day (*B. ruminatus* No. 9).

DULCITE: In no instance was there any action on dulcite.

Time relations: If any change occurred at all it was in the form of gas production, appearing after seven days' incubation, but without the formation of acid (*B. ruminatus* No. 9).

DEXTRIN: Five specimens showed acid formation.

Time relations: Occasionally gas was formed after seven days' incubation (*B. petasites* No. 23).

OTHER BIOLOGICAL PROPERTIES.

Indol formation.—The formation of indol in peptone water was noticed in a few instances, and when present was very decided. There was no regularity of action in relation to the grouping according to sugar fermentation, typically in *B. mesentericus*, *B. ochraceus* (in one example), *B. mesentericus* (in three examples), *B. megatherium* (in one), *B. simplex* (in one), *B. subtilis* (in three).

Nitrites.—The formation of nitrites was equally irregular, and was not always met with in the same examples as was the production of indol. *B. tenuis* (two examples), *B. cretaceus* (two), *B. mesentericus* (two), *B. fusiformis* (one), *B. gastrophilus* (one), *B. vulgatus* (three).

Resistance to ordinary agents.—It was found that the resistance of the spores of these bacilli was remarkably high. This was proved by difficulties in getting rid of the organisms by the ordinary methods of sterilization. The only method of destroying the spores with certainty was by dry heat to 120° C. for a quarter to half an hour. Autoclaving was not successful unless repeated five or six times. As regards the sterilizing of exploratory syringes which had become contaminated with such organisms, it was found that prolonged boiling in carbolic lotion was necessary.

Optimum temperature.—As a rule the organisms flourish best at 30° C., but they will grow rapidly at room temperature. They grow very rapidly in the incubator, the whole surface of the agar being covered within 12 hours in many instances.

DISTRIBUTION IN NATURE.

It is hardly necessary to state that the members of this group are very widespread, but the wide distribution of the *varieties*

named is not so generally understood. Thus we have found them in throat cultures as well as in throat swabs, and we have also obtained variants of *B. mesentericus* in the lymph glands of the groin both in children and adults in the general hospital wards. Excluding technical errors we have come to consider that such organisms may occur in individuals suffering from no disease with which such an organism could have a causal relation.

That false interpretation could be placed on such findings is shown by the report of a "cancer bacillus" by Sheurlen which belonged to this group. There seems little doubt that the greatest precautions will not prevent the discovery of such organisms in the fluid obtained by exploratory puncture of lymphatic glands, whether of the neck or groin. The fact that they have been recovered in this way indicates that the organisms may be found actually in those situations in certain individuals, and that their presence is accidental and not of pathological significance.

A number of the organisms studied were obtained from the intestinal contents of various animals, and were consequently derived from the material ingested by them. Those found in canned eggs may be supposed to have been introduced either after passage through fowl intestine, or by contamination with earth or by manipulation during canning. Others were found in contaminated culture tubes, etc., in the laboratory.

DIAGNOSIS TABLE.

Having dealt with the characters possessed by the different forms demarcated in the literature, and having described the cultural character of numerous examples studied in this laboratory (61 different forms have come under our notice), a few suggestions may be offered as to the best means of proceeding to a diagnosis of such organisms. The characters common to the group are—gram-positive staining,¹ spore formation, liquefaction of milk in a characteristic manner, liquefaction of gelatin,² and defective action on the sugars.³ The motility is variable, and its presence or absence is consequently of no diagnostic value as a group reaction but might

¹ There are one or two exceptions.

² Some forms liquefy gelatin exceedingly slowly.

³ By this is meant the formation of acid without gas, and the feeble extent of the former.

be useful for species-determination. First one notices the appearance on an agar slant. A 24-hour culture will present so marked a growth that it may be observed and provisionally identified till prolonged incubation has shown whether or not pigmentation develops. Such a change may not occur for several days. The agar culture presents appearances which may be grouped as follows:

DIAGNOSIS-SCHEME OF SPORE-BEARERS AND MILK-LIQUEFIERS.*

		GROWTH ON AGAR	
Surface dry	gray-white	dextrose fermenters	<div> <div>motile</div> <div>non-motile</div> </div> <div> <div><i>B. subtilis</i></div> <div><i>B. robur</i></div> </div>
		dextrose plus saccharose fermenters	<div> <div><i>B. ellenbachensis</i></div> <div><i>B. carotarium</i></div> </div>
	yellowish gray chalklike edges feathery	non-fermenters— <i>B. tenuis</i>	
		— <i>B. petasites</i> — <i>B. cretaceus</i> — <i>B. mycoides</i>	
	scanty growth	<div> <div>non-saccharose fermenters</div> <div>non-fermenters—<i>B. gastrophilus</i></div> </div> <div> <div>motile</div> <div>excessively motile—<i>B. asterosporus</i></div> </div>	<div> <div><i>B. tumescens</i></div> <div><i>B. granulatus</i></div> </div>
Surface gummy	minute folds	<div> <div>fatty surface—<i>B. ellenbachensis</i></div> <div>yellow white</div> </div> <div> <div>feebly motile—<i>B. parvus</i></div> <div>very motile—<i>B. fusiformis</i></div> </div>	
	no folds	<div> <div>yellow—<i>B. ochraceus</i></div> <div>white—<i>B. mesentericus</i></div> </div>	
Surface moist	transparent	<div> <div>milk not coagulated—<i>B. sphericus</i></div> <div>milk coagulated</div> </div> <div> <div><i>B. asterosporus</i></div> <div><i>B. oleae</i></div> </div>	
	white woolly edges	— <i>B. butyricus</i> — <i>B. pumilus</i>	
Surface very moist and exuberant	white; prominent folds	<div> <div>yellowish—<i>B. ruminatus</i></div> <div>dirty gray —<i>B. graveolens</i></div> </div>	
		<div> <div>coagulated milk—<i>B. vulgatus</i></div> <div>does not coagulate milk</div> </div> <div> <div><i>B. aterrimus</i></div> <div><i>B. geniculatus</i></div> </div>	
Surface glassy	smooth	<div> <div>non-saccharose fermenter</div> <div>saccharose fermenter—<i>B. mesentericus</i></div> </div> <div> <div><i>B. silvaticus</i></div> <div><i>B. simplex</i></div> </div>	
		<div> <div>does not coagulate milk</div> <div>coagulates milk—<i>B. pumilus</i></div> </div> <div> <div>sugars not affected—<i>B. fusiformis</i></div> </div>	
	retiform— <i>B. mesentericus ruber</i> minute folds — <i>B. teres</i> ocher-colored — <i>B. ochraceus</i> †		

* This table excludes anthrax and its relatives.

† N.B.—The pigment may not develop for several days.

In interpreting this diagnostic table, it must be constantly borne in mind that the characters are not absolutely constant in any one case. It is frequently found that the same organism will change in appearance on prolonged incubation, or, as has already been mentioned, after successive culturing or planting. Moreover,

it is difficult adequately to describe the appearances of some forms from mere lack of suitable adjectives to express them. The wrinkling of the surface, on which much stress is apparently laid, may vary considerably in form, and may be very late in appearing. But with these provisos the classification may be found useful. We have added to the existing types a few others which appeared to be new, and which were very strikingly different in aspect.

CONCLUSIONS.

1. Members of the subtilis and mesentericus group are sufficiently frequently met with as contaminants to make better acquaintance with them advisable.
2. The forms are in many cases interchangeable.
3. Certain varieties may occur in apparently normal human tissues.
4. The differential diagnosis is aided by testing the action of the particular organism upon the various sugar media, of which dextrose and saccharose are the more important.
5. The elaborate nomenclature referred to on p. 212 would be satisfactorily replaced by a classification into *B. subtilis* or *B. mesentericus*, each of which is subdivided into the following types:
 - A. Acidifying dextrose alone.
 - B. Acidifying dextrose and saccharose.
 - C. Acidifying dextrose, saccharose, and mannite.
 - D. Acidifying maltose also.
 - E. Having no action on sugar.
6. An organism would thus be labeled "*B. mesentericus*, type B," if it presented the ordinary features of *B. mesentericus* and presented the typical reactions on sugar.
7. The presence of variants as regards other properties would be specified when necessary.
8. The cardinal features of the group would consist in: gram-positive staining, spore formation, ability to grow at ordinary temperature, liquefaction of milk without coagulation or acid change, feeble or no pathogenicity to laboratory animals.

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